In-Vitro Studies on the Antioxidant Assay Profiling of Root of *Withania somnifera* L. (Ashwagandha) Dunal: Part 2

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Summary

The anti-oxidative activities of six different extracts of *Withania somnifera* (Ashwagandha) root, prepared in a sequential manner starting from non-polar (hexane) to polar (water) solvent, were investigated employing various established *in-vitro* systems that include total antioxidant activity (TAA), total reducing power (TRP), nitric oxide scavenging activity (NOSA) and lipid peroxidation inhibition activity (LPIA). Among all the extracts, methanol extract was found the most potent and additionally, its DNA damage protective efficacy was tested using pRSET-A vector system. Positive correlations were established between total polyphenolic contents (TPC) and various activities strongly suggesting that the observed activities of the extracts may be ascribed to their phenolic compounds that could be responsible, at least partly, for the observed antioxidant activities. Six main compounds *viz.* alkaloids, hydroxybenzene, terpene ansteroid, saponin, organic acids and flavone were identified in methanol extract using thin layer chromatography (TLC) while by employing reverse-phase high pressure liquid chromatography (RP-HPLC) four polyphenols namely epicatechin (3.21 µg/g), quercetin-3-rhamnoside (1.12 µg/g), gallic acid (0.05 µg/g) and rutin hydrate (0.01 µg/g) were identified and quantified in aforementioned extract. Overall, the results of study clearly demonstrated that methanolic extract of Ashwagandha root possesses a marked antioxidant activity.

Key words

reducing potential, free radicals, DNA damage, polyphenols

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Introduction

Physiological metabolites generated in an animal body include numerous oxygen derivatives like superoxide anion, singlet state oxygen and hydroxyl radicals along with peroxides (Halliwell and Gutteridge, 1989). *In vivo*, some of these reactive oxygen species (ROS) play positive roles such as energy production, phagocytosis, regulation of cell growth, intercellular signaling and/or synthesis of biologically important compounds (Halliwell, 1997). However, they may also be very damaging as they can induce oxidation of lipids, causing membrane damage, decreasing membrane fluidity and leading to cancer via DNA mutation (Dasgupta and De, 2007). In recent years, studies on ROS generated oxidative stress and its subsequent ill effects on human health have become a subject of considerable interest.

Human body can be protected from these harmful compounds by its intrinsic enzymatic system, scavengers and antioxidants (Akinpelu et al., 2010). The antioxidants form an intricate network and are capable of preventing oxidative processes by inhibiting the initiation or propagation of an oxidative chain reaction and thereby can prevent many oxidative-stress related diseases (Kumaran and Karunakaran, 2007). But, the natural defence of the body against free radicals is not always sufficient since free radical generation phenomenon increases drastically through radiation, alcoholism, stress and toxins present in food and/or drinking water (Celiktas et al., 2007).

Various plant extracts have attracted interest as sources of natural products and it has been shown that good health can be achieved through the consumption of plants with high antioxidant activities (Dasgupta and De, 2007). Scientific studies have shown that medicinal plants, herbs and fruits with a high content of bioactive compounds and related antioxidant capacity are inversely associated with morbidity and mortality. Some of these medicinal plants are semi-domesticated and mostly grow as weeds (Kumaran and Karunakaran, 2007). Nearly all the civilizations, from ancient times until today, have used plants as a source of medicine. In many developing countries traditional medicine is still the mainstay of health care and most of the drugs and cures come from plants.

The Indian medicinal plant *Withania somnifera* (L.) Dunal (family *Solanaceae*), commonly known as Ashwagandha, is widely used in herbal medicine for stress, arthritis, inflammations, conjunctivitis and tuberculosis (Archana and Namasivayam, 1999; Prakash et al., 2001; Davis and Kuttan, 2002; Gupta et al., 2003; Jayaprakasam and Nair, 2003). The active principles of Ashwagandha are sitoindisides VII-X and withaferin-A that have been shown to exhibit significant antistress and antioxidant effects in rat brain frontal cortex and striatum (Gupta and Rana, 2007b). The medicinal properties of Ashwagandha are attributed mostly to its antioxidant compounds but the detailed report on its antioxidant assay profiling is lacking. The information on the antioxidant potential of Ashwagandha root extracts, using various established *in-vitro* assays, has earlier been published by us (Pal et al., 2011). In continuation, the present manuscript delineates the antioxidant properties of extracts with an emphasis on the DNA damage preventing efficacy of methanolic extract. An attempt has additionally been made to identify and quantify the main individual polyphenolic compounds of methanolic extract using reverse-phase high pressure liquid chromatography (RP-HPLC).

Materials and methods

Preparation of sample extract

The Ashwagandha roots were obtained from the garden of Government Ayurvedic Medical College (GAMC), Mysore, India. The roots were dried; powdered and antioxidant compounds were extracted by adding solvents in increasing order of their polarity, namely hexane (H), chloroform (C), ethyl acetate (EA), acetone (A), methanol (M) and water (W) in a sequential manner. After filtering through folded Whatman No. 1 filter paper, the supernatant in different solvents was recovered and this process was repeated thrice with each solvent. Then, the respective solvents from the supernatant were evaporated in a vacuum rotary evaporator to obtain the yields of different extracts. To check the antioxidant activity, each extract/fraction was dried and redissolved in dimethylsulfoxide (DMSO).

Total antioxidant activity (TAA)

The assay was performed as described by Prieto et al. (1999). In brief, the tubes containing extract and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95°C for 90 min and after the mixture had cooled to room temperature, the absorbance was measured at 695 nm. The antioxidant capacity was expressed as $AO_{0.5\ AU}$ (amount of extract that produces 0.5 absorbance units at selected wavelength). Gallic acid was used as a standard antioxidant.

Total reducing power (TRP)

The reducing power of extract was determined by the method of Oyaizu (1986) with slight modifications. Briefly, different concentrations of extract (1 ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture followed by centrifugation for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (2.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power. The reducing power was expressed as $RP_{0.5\ AU}$ (amount of extract that produces 0.5 absorbance units at selected wavelength). Vitamin C was used as a standard antioxidant.

Nitric oxide scavenging activity (NOSA)

The procedure is based on the principle that sodium nitroprusside in aqueous solution at near neutral pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. In the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations of samples/standard and incubated at room temperature for 150 min. The same reaction mixture without the sample/standard served as the control. After the incubation period, 0.5 ml of Griess reagent [1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine HCl] was added. The

absorbance of the formed chromophore was recorded at 546 nm (Sreejayan and Rao, 1997). The percent scavenging activity was calculated from control without extract under similar conditions. IC_{50} was calculated from linear regression analysis. L-ascorbic acid was used as a standard antioxidant.

Lipid peroxidation inhibition activity (LPIA)

A modified thiobarbituric acid reactive species (TBARS) assay (Ohkowa et al., 1979) was used to measure the lipid peroxide formed using egg yolk homogenates as lipid-rich media (Ruberto et al., 2000). Briefly, egg homogenate (0.5 ml of 10% v/v) and 0.1 ml extract were taken in a test tube and made up to 1 ml with distilled water. Then, 0.05 ml of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and the mixture was incubated for 30 min. It was followed by addition of 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate (SDS). The resulting mixture was vortexed and heated at 95°C for 1 h. After cooling, 5.0 ml of n-butanol was added and the content was centrifuged at 3,000 rpm for 10 min. The absorbance of the upper organic layer was measured at 532 nm. Inhibition of lipid peroxidation (%) was calculated using control without sample under similar conditions. IC_{50} was calculated from linear regression analysis. BHA (butylated hydroxyanisole) was used as a standard antioxidant.

DNA strand break assay

DNA damage protective efficacy of methanol extract was checked using pRSET-A plasmid DNA. Plasmid DNA was oxidized with H_2O_2 + UV treatment in the presence and absence of extract and checked on 1% agarose gel according to Russo et al. (2000) after modifications. In brief, the experiments were performed in 10 µl volume in a microfuge tube containing 350 ng of plasmid DNA in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). H_2O_2 was added at a final concentration of 10 mM with and without methanol extract. The reaction was initiated by UV irradiation and continued for 5 min on the surface of a UV transilluminator at 312 nm under room temperature. After irradiation, the reaction mixture along with gel loading dye was placed on 1% agarose gel for electrophoresis. Untreated plasmid DNA was used as a control. Gel was stained with ethidium bromide and photographed in Gel Documentation system.

Characterization of methanol extract

The methanolic extract being most potent was examined for its major compounds using thin layer chromatography (TLC) analysis. The extract was spotted on silica TLC plates and n-butanol : acetic acid : water (19:0.5:1) was used as the solvent system. The various compounds were identified by spraying different indicators (Tu, 2000; Xin et al., 2008). Initially, the plates were heated at 110°C for 30 min for activation.

The analytical HPLC system consisting of a JASCO high-performance liquid chromatograph coupled with a UV/VIS multi-wavelength detector was employed for the identification and quantification of individual polyphenol of methanolic extract. The mobile phase consisted of 0.1% formic acid (solvent A) and methanol (solvent B). The programme/gradient used was: 85% A/15% B (0 min), 20% A/80% B (55 min), 85% A/15% B (60 min). The flow rate was 0.8 ml/min and the injection volume was 20 µl. Absorbance was monitored at 270 nm and the identification of each polyphenol was based on retention time (Ross et al., 2009).

Results and Discussion

Ashwagandha is a well-known anti-stress and adaptogenic herb. Its anti-inflammatory, anti-tumour, anti-stress, immunomodulatory and hematopoietic properties have been well documented. Traditionally, this herb is consumed to increase life-span and delay ageing. The roots of Ashwagandha contain several alkaloids, withanolides, a few flavonoids and reducing sugars besides rich in iron (Harikrishnan et al., 2008).

The very complex chemistry of oxidation and anti-oxidation processes indicates clearly that a single testing method can't provide the comprehensive picture of the antioxidant potential of a given herb. It is, therefore, a multi-method approach has been advocated to judge completely the antioxidant potential of a sample. The present investigation begins with the extraction of Ashwagandha root compounds in solvents of varied polarity in a sequential manner (non-polar to polar) followed by evaluation of antioxidant activities of all extracts. A number of different assay systems *viz*. TAA, TRP, NOSA, LPIA were included in the investigation. Finally, DNA damage protective efficacy of methanol extract was evaluated and main compounds present in it were identified.

Taking zero percent inhibition in the assay mixture without extract, linear regression equations were developed from a plot between the extract concentration and percentage inhibition of free radical formation/prevention. The resulting equations were employed for the calculation of IC_{50} values (concentration of sample required to scavenge 50% free radicals). The IC_{50} values are inversely related to the activity.

The data pertaining to total polyphenolic contents (TPC), flavonoids, flavonols and tannins of different extract has been published elsewhere (Pal et al., 2011). However, to establish the correlations with antioxidant properties discussed in present manuscript, the data is reproduced here (Table 1).

Table 1. Total polyphenols, flavonoids, flavonols and tannins in different extracts of Ashwagandha root

Extract	TPC	Flavonoid	Flavonols	Tannins
Hexane	4.96±0.2	0.19±0.07	0.11±0.04	1.86±0.09
Chloroform	42.00±3.4	0.52±0.09	0.29±0.06	19.76±1.5
Ethyl acetate	61.09±5.4	0.82±0.1	0.45±0.07	29.86±2.1
Acetone	45.31±3.8	0.49±0.08	0.28±0.07	27.24±1.9
Methanol	88.58±6.7	0.85±0.14	0.48±0.08	37.03±2.4
Water	66.72±2.9	0.38±0.04	0.21±0.05	16.96±1.1

Total antioxidant activity (TAA)

The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acidic pH with a maximum absorbance at 695 nm. The assay is successfully used in the quantification of vitamin E in seed. Since the assay is very simple and independent of other

Table 2. Regression equations for TAA of different extracts of Ashwagandha root

Extract	Regression equation	Correlation coefficient (*r*)
Hexane	y = 0.1161x + 0.0689	0.9664
Chloroform	y = 0.0006x - 0.0290	0.9880
Ethyl acetate	y = 0.0004x + 0.0382	0.9728
Acetone	y = 0.0004x + 0.0513	0.9663
Methanol	y = 0.0013x - 0.0327	0.9834
Water	y = 0.0004x + 0.0521	0.9664

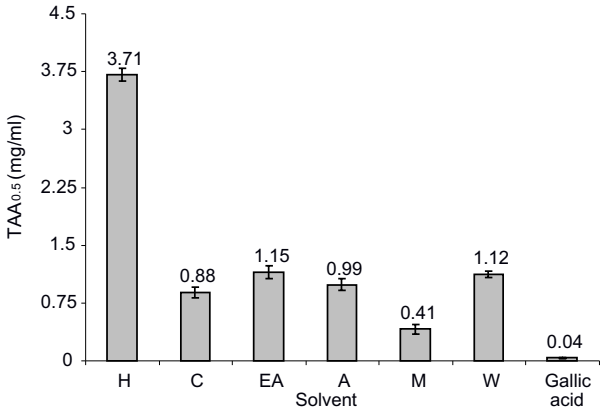

Figure 1. Comparison of TAA activity of different extracts of Ashwagandha root

Table 3. Correlation coefficient (*r*) between antioxidant contents and antioxidant activities

Antioxidant activity	Antioxidant contents			
	TPC	Flavonoids	Flavonols	Tannins
$AO_{0.5\ AU}$	0.747	0.549	0.552	0.765
$RP_{0.5\ AU}$	0.841	0.563	0.557	0.741
NO	0.833	0.596	0.603	0.858
LPIA	0.844	0.511	0.505	0.703

antioxidant measurements commonly employed, its application has been extended to plant extracts (Prieto et al., 1999).

An increase in absorbance indicates an increase in TAA. The TAA of different extracts was measured and expressed in terms of $AO_{0.5\ AU}$. Various regression equations were generated based on the dose dependent response of extracts and are shown in Table 2. A comparison of $AO_{0.5\ AU}$ of different extracts is shown in Fig. 1 wherein it is obvious that methanol extract possesses maximum TAA while it is the least for hexane extract. Correlation coefficients (*r*) were calculated for different antioxidant contents of these extracts and a maximum *r* was observed with tannins (76.5%) followed by TPC (74.7%) indicating that TAA, to certain extent, can be attributed to tannins and TPC (Table 3). Very weak correlations were observed with flavonoids and flavonols.

The antioxidant activity of plant extracts has been attributed to various mechanisms including prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxide, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging activity (Kumaran and Karunakaran, 2007).

Total Reducing Power (TRP)

The antioxidant activity of plant extracts have been correlated with their reducing powers (Tanaka et al., 1988), which in turn is generally associated with the presence of reductones (Duh, 1998). The reductones exert antioxidant action by breaking the free radical chains, via hydrogen atom donation (Gordon, 1990). Additionally, reductones are also reported to prevent peroxide formation, by reacting with certain precursors of peroxide (Kumaran and Karunakaran, 2007). Therefore, the reducing power of an extract may serve as a significant indicator of its potential antioxidant activity (Hsu et al., 2006).

In the present study, TRP of all extracts was assayed and linear regression equations were generated based on dose dependent response. The regression equations are summarized in Table 4 while a comparison of TRP in terms of $RP_{0.5\ AU}$ is presented in Fig. 2. The methanolic extract was found to possess maximum TRP with $RP_{0.5\ AU}$ value of 0.32 mg/ml. Ethyl acetate and water extracts also showed fairly good TRP with almost equal $RP_{0.5\ AU}$ values while hexane extract exhibited the least of TRP (4.5 mg/ml). The correlation coefficient of TPC with TRP was 84.1% (Table 3) that clearly indicates that the observed re-

Table 4. Regression equations for TRP of different extracts of Ashwagandha root

Extract	Regression equation	Correlation coefficient (*r*)
Hexane	y = 0.1133x - 0.0176	0.9836
Chloroform	y = 0.3200x + 0.0300	0.9816
Ethyl acetate	y = 0.0010x - 0.0167	0.9890
Acetone	y = 0.4336x + 0.0448	0.9742
Methanol	y = 0.0015x + 0.0238	0.9840
Water	y = 0.0010x - 0.0297	0.9855

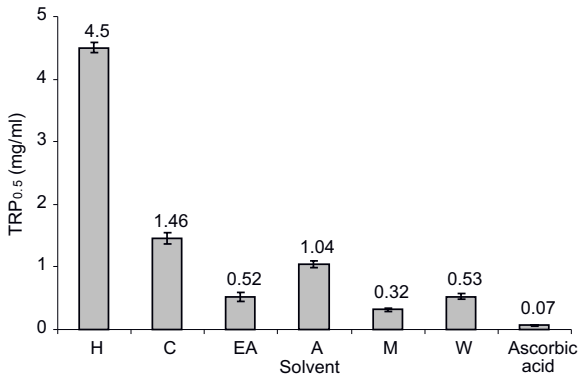

Figure 2. Comparison of TRP of different extracts of Ashwagandha root

ducing potential of extracts is primarily due to their polyphenolic compounds. A fair degree of correlation was found with tannins (74.1%) while the least correlation was found with flavonoids (56.3%) and flavonols (55.7%). The results indicate that TRP of extracts significantly contribute towards the observed antioxidant effect.

Nitric oxide scavenging activity (NOSA)

Nitric oxide or reactive nitrogen species (RNS), formed during their reaction with oxygen or with superoxides, such as NO_2, N_2O_4, N_3O_4, $NO3^-$ and $NO2^-$ are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components (Moncada et al., 1991). Incubation of solutions of sodium nitroprusside in PBS (sodium perborate) at room temperature resulted in linear time-dependent nitrite production that was reduced by all the extracts of Ashwagandha root. The regression equations were developed using the dose-dependent response of all the extracts that are listed in Table 5. The IC_{50} values of various extracts, derived using these equations, are shown in Fig. 3. The methanolic extract was found to be the most potent as reflected by its the lowest IC_{50} value (3.81 mg/ml). A good correlation was observed with tannins (85.8%) and TPC (83.3%) indicating that NOSA can be attributed to the presence of tannins and TPC in extracts. The obtained data indicates that the plant extract may be of considerable interest to arrest the chain of reactions initiated by excess generation of NO (nitric oxide) that are detrimental to the human health.

Table 5. Regression equations for NOSA of different extracts of Ashwagandha root

Extract	Regression equation	Correlation coefficient (*r*)
Hexane	y = 4.7931x - 0.3238	0.9894
Chloroform	y = 6.5943x + 0.9286	0.9784
Ethyl acetate	y = 9.6000x + 0.7000	0.9852
Acetone	y = 9.8857x + 2.6952	0.9809
Methanol	y = 12.2960x + 3.1250	0.9771
Water	y = 9.0743x + 0.0714	0.9816

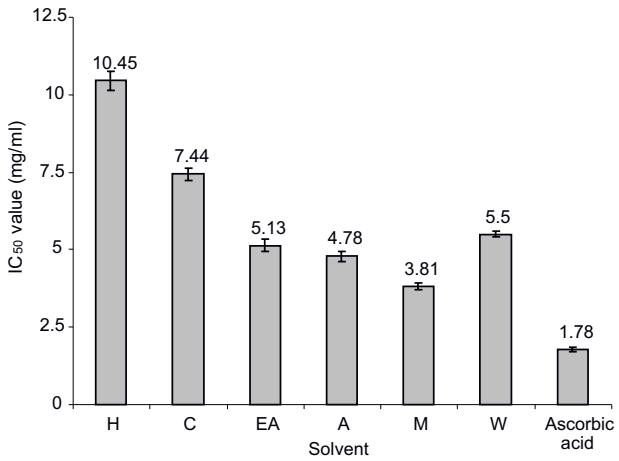

Figure 3. Comparison of NOSA of different extracts of Ashwagandha root

Lipid peroxidation inhibition activity (LPIA)

Free radical scavenging is a generally accepted mechanism for antioxidants to inhibit lipid peroxidation. The inhibition of lipid peroxidation induced by $FeSO_4$ in egg yolk was assayed by measuring the lipid peroxidation products such as TBARS. All the extracts of Ashwagandha showed inhibition of lipid peroxidation in a dose dependent manner. The regression equations developed for the calculation of IC_{50} values of all extracts is shown in Table 6 and a comparison of IC_{50} values is shown in Fig. 4. Results showed that all the extracts inhibited TBARS formation in a dose dependent manner with maximum activity shown by methanol (0.49 mg/ml) followed by water extract (0.53 mg/ml). It has been suggested that antioxidant activity of an extract is its ability to delay the onset of auto-oxidation by scavenging reactive oxygen species, or its ability to act as chain-breaking antioxidants by inhibiting the propagation phase of lipid auto-oxidation (Nawar, 1996).

Table 6. Regression equations for LPIA of different extracts of Ashwagandha root

Extract	Regression equation	Correlation coefficient (*r*)
Hexane	y = 15.526x + 2.7524	0.9867
Chloroform	y = 0.0426x + 0.2905	0.9854
Ethyl acetate	y = 0.0674x + 1.7071	0.9890
Acetone	y = 0.0542x - 0.3667	0.9897
Methanol	y = 0.1026x - 0.1357	0.9881
Water	y = 0.0963x - 0.9648	0.9908

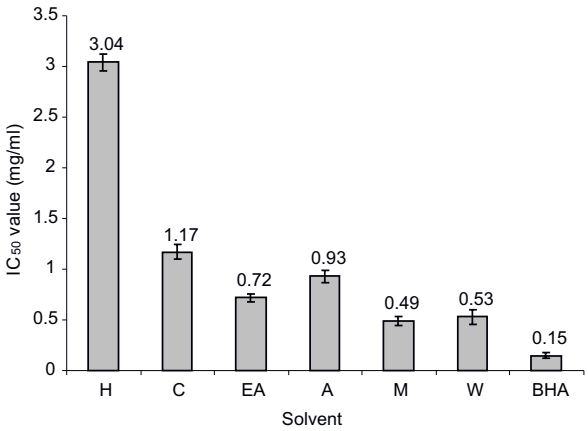

Figure 4. Comparison of LPIA of different extracts of Ashwagandha root

The correlation coefficient relationships of LPIA with different antioxidant contents are shown in Table 3 and these were found maximum with TPC (84.4%) and tannin (70.3%). These results indicate the contribution of TPC and tannin towards LPIA of different extracts. The obtained correlations are particularly useful when the content of antioxidant is known because it allows the estimation of antioxidant activities instead of their experimental determination.

DNA damage protective efficacy of methanol extract

The methanol extract, being the most potent fraction in terms of its antioxidant and reducing potential, was tested for its protective effect on H_2O_2 + UV-induced damage on pRSET-A plasmid DNA. Fig. 5 shows the electrophoretic pattern of DNA after UV-photolysis of H_2O_2 in the presence (20-60 µg) and absence of extract. The DNA derived from pRSET-A plasmid showed two bands on agarose gel electrophoresis (lane 1) wherein the faster moving prominent band corresponded to the native supercoiled circular DNA (ScDNA) and the slower moving faint band corresponded to the open circular form (OcDNA). The UV irradiation of DNA in the presence of H_2O_2 (lane 2) resulted in almost complete degradation of ScDNA to give a smear indicating that hydroxyl radical generated from UV-photolysis of H_2O_2 caused DNA degradation. Although both superoxide and peroxide radicals are potentially cytotoxic, most of the oxidative damage in biological systems is caused by the hydroxyl radical that is generated by the reaction between superoxide and peroxide in the presence of metal ions (Guitteridge, 1984). The addition of Ashwagandha root extracts to the reaction mixture provided significant protection to the damage of ScDNA in a dose dependent manner (lanes 3-5).

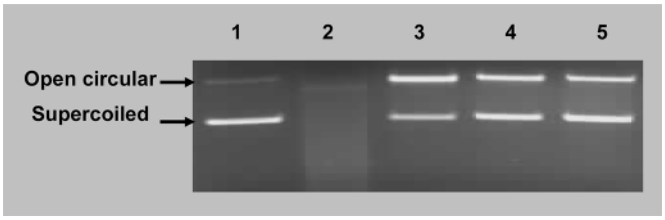

Figure 5. Electrophoretic pattern of pRSET-A DNA after UV-photolysis of H_2O_2 in the presence or absence of Ashwagandha root methanolic extract. Lane1: control, lane 2: treated, lane 3: Ashwagandha extract (20 µg), lane 4: Ashwagandha extract (40 µg) and lane 5: Ashwagandha extract (60 µg).

Characterization of methanol extract

Since the methanol extract of Ashwagandha root was found the most potent for antioxidant activity, its main compounds were identified using TLC assays and the results are summarized in Table 7. Six main compounds namely alkaloid, hydroxybenzene, terpene ansteroid, saponin, organic acids and flavone were identified that may be considered responsible for antioxidant activity of extract.

An attempt was made to identify the main individual polyphenolic compounds present in methanolic extract of Ashwagandha root. By employing RP-HPLC, four polyphenols namely epicatechin (3.21 µg/g), quercetin-3-rhamnoside (1.12 µg/g), gallic acid (0.05 µg/g) and rutin hydrate (0.01 µg/g) were identified and quantified (Fig. 6) while few remained unidentified due to the lack of standards. Quantification was done through 'external standard method' *via* calibration with standards. The content of polyphenols detected in the analyzed sample is shown in Table 8.

Table 7. Main compounds of methanolic extract of Ashwagandha root

Indicator	Compounds	Color
Iodine (fumes)	Alkaloid	Brown
Ferric trichloride in water (0.1%)	Hydroxybenzene	Purple
Acetic anhydride : Sulphuric acid (100:1)	Terpene ansteroid	Prunosus
Phosphomolybdic acid in ethanol (1%)	Saponin	Dark blue
10% NaOH	Flavone	Yellow
Bromophenol blue in ethanol (0.1%)	Organic acid	Dark yellow

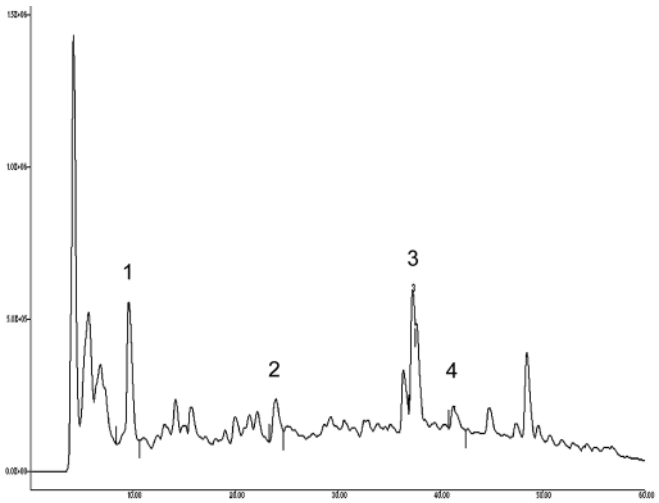

Figure 6. RP-HPLC chromatogram of polyphenols of methanolic extract of Ashwagandha root (1= gallic acid, 2= epicatechin, 3= rutin hydrate, 4= quercetin-3-rhamnoside).

Table 8. Individual polyphenol of methanolic extract of Ashwagandha root

Polyphenols	Retention time (min)	Amount (µg/g)
Gallic acid	10.29	0.05
Epicatechin	24.05	3.21
Rutin hydrate	37.33	0.01
Quercetin-3-rhamnoside	40.61	1.12

Conclusion

In this study, using various established *in-vitro* assay systems, the antioxidant potential of Ashwagandha root extracts was evaluated. In general, the methanol extract showed strong TAA, TRP, NOSA, and LPIA. The results clearly confirmed the anti-oxidative and free radical scavenging activity of the extracts. In addition, DNA damage preventive efficacy of methanol extract, the most potent, was also evaluated using pRSET-A plasmid DNA. Six main compounds, namely alkaloid, hydroxybenzene, terpene ansteroid, saponin, organic acids and flavone were identified using TLC while by employing RP-HPLC, four polyphenols, namely epicatechin (3.21 µg/g), quercetin-3-rham-

noside (1.12 µg/g), gallic acid (0.05 µg/g) and rutin hydrate (0.01 µg/g) were identified and quantified in the methanol extract. The profound protective effect of Ashwagandha extract against DNA damage, free radical scavenging and inhibition of lipid peroxidation may explain its use in daily life and possible health benefits. Further work on the isolation and purification of Ashwagandha root compounds and evaluation of their performance enhancing properties are in progress.

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